Environmental niche separation promotes coexistence among ecologically similar zooplankton species—North Sea copepods as a case study

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1 **Environmental niche separation promotes coexistence among ecologically similar zooplankton**
2 **species – North Sea copepods as a case study**

3
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9
10 ***Abstract***

11 Marine zooplankton are among the most diverse and abundant organisms on earth. Despite a great
12 wealth of knowledge and research on their ecology, the processes promoting co-existence and
13 maintaining their high diversity worldwide are poorly known. In order to understand the processes
14 underpinning coexistence among marine zooplankton, we investigated the existence and degree of
15 niche separation within two pairs of taxonomically and ecologically related species of copepods
16 belonging to the widespread, abundant genera *Calanus* and *Oithona*. We compared the spatial
17 variation in species abundances to the abiotic and biotic environment using a multi-model approach
18 and vertically resolved survey data on zooplankton composition, abundances and environmental
19 conditions in the North Sea. Our results demonstrate pronounced spatial differences between
20 species in each pair, primarily in their vertical abundance distributions. These differences can
21 largely be explained by different preferences for temperature and salinity. This supports the
22 occurrence of environmental niche separation in marine zooplankton and highlights its role as a
23 mechanism reducing inter-specific competition and promoting coexistence.

24
25 **Key words:** niche differentiation, coexistence, competition, climate, zooplankton, random forests

26

27 ***Introduction***

28 Niche separation (or the analogous terms niche segregation and niche differentiation) is commonly
29 regarded as the key process favouring coexistence among ecologically and functionally similar
30 species of plants and animals (MacArthur 1958; Pianka 1974; Ben-David et al. 1996; Silvertown
31 2004; Angert et al. 2009). It therefore contributes to the process of speciation and the maintenance
32 of biodiversity at large (Chesson 2000). Niche separation generally occurs through a reduction in
33 competition for limiting resources or living space, which, if left unresolved would inevitably lead to
34 the competitive exclusion of weaker competitors and a loss of biodiversity (Hardin 1960). The ways
35 in which species can reduce inter-specific competition are numerous (e.g. consuming different
36 foods, or using different parts of the physical environment) but all serve to modify the resource
37 requirements of competing species so as to reduce, or minimize overlap between their fundamental
38 niches (Hutchinson 1957).

39
40 Although conceptually well-understood, investigating niche separation in the field is challenging,
41 primarily due to the long time-scales over which natural selection and niche separation occurs.
42 Instead, we typically have to resort to studying what is sometimes termed “the ghost of competition
43 past” (Connell 1980), the evolutionary end-result of competitive exclusion and niche separation.
44 This end-result is often manifested in slight but important differences in the niches of closely
45 related and competing species, for instance in terms of their diet requirements, the type of predators
46 they are exposed (and adapted) to, as well as their preferences for certain physical habitats
47 (MacArthur 1958; Crowder 1986; Steiner et al. 2007; Wilson et al. 2015). Alternatively, niche
48 separation may be studied by investigating short-term responses of natural communities to invasive
49 species (Mooney and Cleland 2001; Mack et al. 2000; Lindegren et al. 2012), leading to either local

50 extinction through competitive exclusion, or coexistence mediated through adaptation (behavioural,
51 morphological or physiological).

52

53 Marine zooplankton constitute among the most diverse, abundant and ecologically important
54 organisms on earth (Verity and Smetacek 1996), directly or indirectly contributing to maintaining
55 key ecosystem functions and services essential for human well-being. These services include for
56 instance food provisioning by acting as key prey for many commercially important fish species
57 worldwide and climate regulation by affecting carbon sequestration through increased downward
58 flux of organic matter via excretion and respiration at depth (Ducklow et al. 2001; Steinberg et al.
59 2002; Jónasdóttir et al. 2015; Visser et al. 2017). Among marine zooplankton, copepods are a
60 dominant and generally well-studied group of organisms, especially in terms of their physiology
61 (e.g. rates of consumption, respiration and metabolism), behaviour (e.g. modes of feeding, mating
62 and predator avoidance) and their role in marine food webs as both predators of phytoplankton and
63 prey for higher trophic level predators (Möllmann et al. 2003; Lindegren et al. 2011; Blenckner et
64 al. 2015). However, the underlying processes promoting co-existence and maintaining their high
65 diversity worldwide are largely understudied with relatively few related examples from the marine
66 literature (e.g. Poulet 1978; Katechakis et al. 2004; Helaouet and Beaugrand 2007).

67

68 The marine copepods *Calanus finmarchicus*, *Calanus helgolandicus*, *Oithona atlantica* and
69 *Oithona similis* represent two pairs of widespread and ecologically important zooplankton species
70 in the North Atlantic. Despite their high abundances the *Oithona* species are largely understudied
71 (Galliene 2001) and often excluded from comparative studies (McGinty et al. 2018), primarily due
72 to undersampling by most standard zooplankton sampling methods. In contrast, the *Calanus*
73 species are very well studied and have attracted considerable attention in the literature (Bonnet et al.

2005; Helaouet and Beaugrand 2007; Jónasdóttir and Koski 2011; Melle et al. 2014). Both pairs coexist in the North Sea despite large similarities in the morphological, physiological and behavioural traits within each genus (Fleminger and Hulsemann 1977; Bucklin et al. 1995). *C. finmarchicus* and *C. helgolandicus* are large (> 2mm), filter-current feeding copepods that in the North Sea constitute key prey for early-life stages and adults of commercially important fish species, e.g. the Atlantic cod (Beugrand et al. 2003) and Lesser sandeel (VanDeurs et al. 2009). The *Oithona* species are considerably smaller (~0.5 mm) ambush feeders on ciliates, detritus and phytoplankton. Consequently, they are thought to serve as an important link of energy from primary producers to fish larvae (Nielsen and Sabatini 1996). Interestingly, these copepod species show pronounced temporal fluctuations and long-term shifts in their relative dominance, particularly between *C. finmarchicus* and *C. helgolandicus*. These shifts have been related to pronounced changes in hydrographic conditions, linked to the Atlantic Multi-decadal Oscillation (Beaugrand 2009). This suggests that closely related species may exhibit opposite affinities for and responses to environmental conditions (Bonnet et al. 2005; Beaugrand 2009; Helaouet and Beaugrand 2007; Wilson et al. 2016). Although characterized by different temporal dynamics, as well as biogeography in the Northern Atlantic, the North Sea constitutes a key area for all these species where they co-occur in high abundances.

In order to understand the processes favouring coexistence among marine zooplankton in general and copepods in particular, we investigated the degree of niche separation using the two abovementioned pairs of highly abundant and widely distributed *Calanus* and *Oithona* species in the North Sea as a case study. Our approach is to identify and quantify differences in habitat preferences and resource use that we assume originated as a result of niche separation in response to

past competition. We hypothesize that any spatial differences in their abundance distributions are due to different responses to the same set of underlying drivers.

Material and methods

Study area and data collection

Sampling was carried out along transects in three areas of the North Sea (Fig. 1; Fig. S1): a) Transect A (Dogger bank) covered stations from the deep central North Sea up along the flanks of Dogger Bank. The transect was sampled for 4 years; 13 August 2002 (2 stations), 19 August 2003 (3 stations), 28 July- 2 August 2005 (4 stations) and 5-7 September 2007 (12 stations), b) Transect B (Buchan) was sampled 8-9 September 2007 and consisted of 10 stations from coast off Buchan towards deeper areas, and c) Transect C (Hanstholm) was positioned in the eastern North Sea, off the Danish coast, with 6 stations sampled 6-7 May 1997 and 8 stations sampled 11-12 September 2007. At each station, hydrographic profiles of temperature, salinity, fluorescence, oxygen and irradiance were measured from surface to 400 m depth or to 5 m above the bottom by a Seabird SBE11 (9+) CTD, with mounted Biospherical QSP-2300L PAR Sensor, SBE 43 Dissolved Oxygen Sensor, and Turner Cyclops-7 Fluorometer. Following the CTD cast zooplankton were sampled. In 2007 the sampling was carried out using a 0.25 m² opening-closing net (Multinet®). The gear was lowered to 5-10 m above bottom and subsequently towed vertically, opening five 53 micron meshed nets at every 10 m to the surface. When depth was more than 50 m, a consecutive haul was carried out at same position to avail samples from all possible 10 meter strata towards the bottom. In 1997, 2002, 2003 and 2005 zooplankton was sampled with a submersible pump. The water was pumped directly into an attached 30 µm plankton net protected by an outer 200 µm mesh net bag. The submersible pump (Homa, H-500, with the plankton net fitted to the outlet) was lowered to a pre-determined depth and turned on (flow 1.2 m³ min⁻¹). In 1997 it was raised within 10 to 20 m wide

121 depth strata before turned off, while it the other years sampled for 3 minutes at depth. In 2002
122 samples were taken at 10 m depth intervals, while at 5 m intervals in 2003 and 2005. The nets were
123 washed, contents transferred to containers and every sample preserved in 4% borax buffered
124 formaldehyde. In the laboratory each sample was subsampled to different degrees, and from each
125 subsample the zooplankton were enumerated, identified to lowest taxonomic levels and length
126 measured, copepods to their cephalothorax lengths. While the sampling gear was not the same
127 during all cruises, the difference should not affect the analysis in this study. The comparisons are
128 done between species sampled with the same gear at the same time. Additionally, the mesh sizes in
129 both sampling devices were fine (30 and 53 μ m) to successfully capture the smaller *Oithona*, and the
130 volume filtered was well monitored for both devices minimizing error in estimates between devices.

131

132 **Statistical analysis**

133 Three complementary statistical tools were used to examine and compare the spatial distribution
134 patterns of species abundances in relation to a number of drivers, representing their environmental
135 niches in terms of: (i) temperature, (ii) salinity, (iii) Chlorophyll a (chl a; a proxy for food
136 concentration); (iv) and the intensity of photosynthetically active radiation (PAR; a proxy for
137 predation risk), each measured at any given transect, station, depth and time. In addition, transect
138 and life-stage were included as factors in the analyses to account for any differences in the mean
139 abundances between areas and between adult males, females and juveniles (copepodites) that
140 cannot be attributed to the environmental conditions. The same set of explanatory variables was
141 used as covariates across species and statistical analyses, including: (i) Generalized Additive
142 Models (GAMs), (ii) random forests (RFs); (iii) and gradient boosting machines (GBMs). All
143 variables had a Variance Inflation Factor (VIF) <3, indicating a negligible degree of collinearity
144 between predictors. After model fitting the resulting statistical relationships and derived response

145 curves between species abundances and the environmental variables, as well as the relative
146 importance among these drivers were compared across methods to assess the sensitivity and
147 robustness of the results and the characterization of species niches to the choice of methodology.
148 Furthermore, we performed a k-fold cross validation analysis (Picard and Cook 1984) to enable a
149 formal comparison of prediction skill across methods by fitting the set of models to randomly
150 selected subsets of the data (amounting to 75% of the observations). We then assessed the
151 predictive accuracy of each model by comparing the observed values with the predicted estimates
152 for the remaining test data sets for each species. The cross-validation analysis was repeated 100
153 times (each time with a new random and serially independent draw) in order to assess the range of
154 uncertainty associated with the predictions (i.e., reflected by the mean square errors (MSE) of
155 model predictions. All statistical analyses were conducted using the R software, version 3.1.2 (R
156 core Team 2012) using the following packages: “mgcv” (Wood 2017), “randomForest” (Liaw and
157 Wiener 2002) and “gbm” (Greenwell et al. 2018).

158

159

160 **Generalized Additive Models**

161 Generalized Additive Models (GAMs; Hastie and Tibshirani 1990; Wood 2017) provide a suitable
162 and commonly used tool to examine non-linear dependencies in ecological data. The following
163 formulations with natural log-transformed species abundance estimates as responses were used:

$$\begin{aligned} 164 \log(N) = & a + s(\text{Temperature}) + s(\text{Salinity}) + s(\log(\text{Chl } a)) + s(\log(\text{PAR})) + \text{transect} + \\ 165 & \text{stage} + e \end{aligned}$$

166 where a is the intercept, s the thin plate smoothing function and ε the error term. Although the
167 number of regression splines is optimized (and penalized) by the generalized cross validation
168 criterion (GCV), the degrees of freedom of the spline smoother function (s) was further constrained

169 to three knots ($k=3$) to allow for potential nonlinearities, but restrict flexibility during model fitting.
170 Finally, we applied backwards model selection based on the GCV and partial F -tests to identify the
171 best possible set of predictors.

173 **Random forests**

174 Random forests (RFs) are a machine learning tool comprising ensembles of decision trees that rely
175 on *bagging* (i.e. bootstrap aggregation). They are used for both regression (as in our study) and
176 classification problems (Breiman 2002). Individual regression trees within the random forest are
177 trained on randomly selected subsets of the data. In each tree, the data is recursively partitioned, at
178 each instance (i.e. node) based on the most strongly associated predictor. At each node in a tree, a
179 random subset of predictors is evaluated for partitioning. The final tree prediction is given by the
180 average value of the data within each branch of the tree. The final forest prediction is obtained by
181 averaging predictions across all trees in the forest. RFs are capable of reproducing complex
182 nonlinear shapes in single and multiple dimensions, making them suitable for ecological
183 applications in which complex shapes are to be expected. In our study, we used the fitted RFs to
184 estimate the nonlinear partial effects of individual variables, which are obtained by averaging out
185 the effects of all other predictors on the quantity of interest. We predicted $\log(\text{abundance})$ with all
186 other variables used in the GAM model above. However, the random forest has fewer constraints
187 and is able to capture interactions between variables that cannot easily be achieved with additive
188 models.

190 **Gradient boosting machines**

191 Gradient boosting machines (GBMs) are a machine learning tool that we use here with decision
192 trees (though they may be constructed with other types of models) (Freund and Schapire 1997;

193 Friedman 2001). When used with decision trees, they differ from RFs primarily in using *boosting*
194 as an ensemble technique instead of bagging. Unlike the bootstrap-based bagging method, boosting
195 works sequentially. Individual decision trees are fit to the residuals of the previous tree till a
196 stopping criterion is reached (to prevent overfitting). Due in part to this sequential nature, GBMs
197 are more sensitive to overfitting problems than RFs but may achieve higher performance.

198

199 **Results**

200 The species demonstrate distinct vertical distribution patterns, where *C. finmarchicus* and *O.*
201 *atlantica* show higher abundances at lower depths (below 45 meters) while *C. helgolandicus* is
202 more evenly distributed throughout the water column and *O. similis* shows a clear preference for
203 surface waters (Fig. 2; Fig. S1-S2). The averaged vertical distribution patterns are largely consistent
204 across life-stages (Fig. S2), except for lower abundances of *C. helgolandicus* juveniles
205 (copepodites) in surface waters compared to adults (divided into males and females), and vice versa
206 for *O. atlantica*. Temperature and salinity were strong abiotic predictors of abundances in all
207 species and methods, illustrated by their high variable importance scores based on RFs and GBMs
208 (Table 1). In addition, Chl *a* proved to be a good predictor. In contrast, PAR had low importance
209 scores and was found to be statistically insignificant in the GAMs. It was therefore excluded during
210 model selection and was omitted as a predictor in the final GAMs (Table S1-S5). In addition to
211 these predictors, the factors transect and life-stage were significant and generally of high relative
212 importance for all species (Table 1; Table S2-S5). The different intercepts highlights regional
213 variability in mean log(abundance) between different areas of the North Sea, as well as between
214 juveniles and adults (separated into males and females) (Fig. S1-S2). Although interesting in its
215 own right, these differences primarily reflect well-known population demographics (i.e., higher
216 number of juveniles compared to adults) and sex specific mortality rates (i.e., higher predation

217 mortalities on males compared to females), giving rise to a skewed sex ratio among copepods (Hirst
218 et al., 2010). Since the primary focus of this study is on potential differences in environmental
219 niches among closely related species we will not discuss and further elaborate on the underlying
220 mechanisms explaining differences in abundances within each species related to these factors. The
221 methods explained a high degree of observed variances in log(abundance) across species, ranging
222 from 36% (*C. helgolandicus*) to 78% (*O. similis*) (Table S2), with RFs generally outperforming
223 GAMs and GBMs in terms of explained variance and mean squared errors of model predictions on
224 test data not included during fitting/training (Fig. 3). Finally, standard model diagnostics for the
225 GAMs demonstrate normally distributed residuals for all species (Fig. S3-S6).

226
227 The differences in the spatial abundance distributions between species are reflected by different
228 responses to the identified drivers, largely representing their environmental niches in terms of
229 temperature and salinity. In terms of temperature, *C. finmarchicus* and *C. helgolandicus* both
230 demonstrate non-linear response curves that are roughly hump-shaped (Fig. 4a). The patterns are
231 fairly consistent across methods, but with RFs and GBMs suggesting a more pronounced affinity of
232 *C. finmarchicus* for colder water than that identified using GAMs (Fig. 2b). The *Oithona* species
233 also demonstrate different responses to temperature that are identified consistently across methods,
234 illustrated by a generally positive saturating relationship of *O. similis* compared to a declining
235 temperature response of *O. atlantica* (Fig. 4c, d). In terms of salinity, different responses between
236 species in each genus are also evident, characterized by non-linear increasing and decreasing
237 relationships for *C. finmarchicus* and *C. helgolandicus*, respectively (Fig. 5a, b). Likewise, *O.*
238 *atlantica* and *O. similis* show opposite salinity responses (Fig. 5c, d), indicating an affinity for high
239 and low salinities respectively. In contrast to temperature and salinity, all species seem to respond
240 in the same way to our proxy for food availability, illustrated by a similar positive linear response to

241 Chl *a* (Fig. 6). The response curves are consistent across methods, except for *C. finmarchicus* and
242 *C. helgolandicus* where the GAMs show no statistically significant relationship (Fig. 6a). In order
243 to summarize the responses of the species to the entire set of environmental drivers we
244 characterized the environmental niches of the species based on GAM predictions of
245 log(abundances) across the entire range of observed temperature, salinity and Chl *a* values. The
246 model predictions show clear differences in the environmental niches of species within the same
247 genera. This is illustrated by limited overlap of predicted log(abundances) in a 3-D niche space
248 represented by temperature, salinity and Chl *a* (Fig. 7). In general, *C. finmarchicus* and *O. atlantica*
249 prevail at low temperatures and high salinities and vice versa for *C. helgolandicus* and *O. similis*.

250

251 ***Discussion***

252 Niche separation is a key process reducing inter-specific competition and thereby facilitating
253 coexistence and the maintenance of biodiversity at large (MacArthur 1958; Pianka 1974; Chesson
254 2000; Silvertown 2004). On the basis of vertically resolved survey data and three complementary
255 statistical approaches we have demonstrated pronounced and methodologically robust differences in
256 environmental niches in two pairs of closely related species of marine copepods. Both pairs coexist
257 in the North Sea, despite large similarities in their morphological, physiological and behavioural
258 traits (Fleminger and Hulsemann 1977; Bucklin et al. 1995). The niche separation could largely be
259 explained by different responses of the species within each genus to temperature and salinity.

260

261 The different responses to temperature highlight a separation between species adapted to colder or
262 warmer waters. This is typically reflected by distinct biogeographic patterns characterized by either
263 a sub-arctic or a boreal distribution, as has been shown for *C. finmarchicus* and *C. helgolandicus* in
264 the North Atlantic (Bonnet et al. 2005; Helaouet and Beaugrand 2007). However, species may not

only differentiate across latitude and longitude but also separate in terms of their vertical distributions. The North Sea is highly stratified, especially during summer when high temperatures and low wind stress suppress mixing and promote a vertical separation of water masses with markedly different physical properties (Nielsen et al. 1993). The vertical separation of water masses is mirrored in the abundance distributions of *O. atlantica* and *O. similis* showing different preferences for deeper and shallow waters, respectively. This in turn is reflected by their opposite temperature responses with peak abundances observed at either the cold or warm end of the temperature range. In contrast to *Oithona*, the *Calanus* species demonstrate a less pronounced vertical separation of abundances, but with *C. finmarchicus* generally preferring deeper waters compared to a more uniform depth distribution of *C. helgolandicus*. The lower degree of vertical separation among *Calanus* is reflected by more similar temperature response for both species. However, their humped-shaped response curves differ in terms of their optima and spread. This is highlighted by a preference for warmer temperatures within a rather narrow temperature range for *C. helgolandicus* and a broader temperature range with a preference for colder temperatures for *C. finmarchicus*. The derived thermal niches support previous observational studies and reviews demonstrating peak abundances of *C. helgolandicus* and *C. finmarchicus* at temperatures $\geq 13^{\circ}\text{C}$ and $< 10^{\circ}\text{C}$, respectively (Bonnet et al. 2005; Helaouet and Beaugrand 2007; Jónasdóttir and Koski 2011; Melle et al. 2014; McGinty et al. 2018) and corresponds well to available meta-analysis across zooplankton taxa showing a general concave relationship with temperature (Forster et al. 2011). The broader temperature range of *C. finmarchicus* allows it to tolerate a larger range of environments and consequently occupy a considerably larger distribution area, as evidenced by its widespread biogeography across the North Atlantic (Helaouet and Beaugrand 2007; Melle et al. 2014). The different temperature responses likely reflect different thermal tolerances of species within each genus, although the link between physiological tolerance and abundance is complicated

289 by the influences of resource availability, interactions with other species, and density-dependent
290 processes. Furthermore, it should be noted that the vertical distributions presented here are based
291 primarily on summer samples (July-September). This is the period when *C. finmarchicus* is
292 preparing for overwintering by accumulating lipids and initiating a descent to deeper waters
293 (Johnson et al. 2008; Melle et al. 2014; Krumhansl et al. 2018). Hence, onset of diapause could
294 contribute to the deeper distribution of the species and partly explain the affinity of *C. finmarchicus*
295 for lower temperatures. However, Jónasdóttir and Koski (2011) demonstrated that females of the
296 same North Sea population were still reproductively active and feeding in the deeper cooler waters
297 during July-August. Hence, we believe that the influence of diapause on the derived thermal
298 response of *C. finmarchicus* is relatively marginal.

299
300 In contrast to temperature, the pronounced differences in the species responses to salinity are more
301 difficult to explain by any direct physiological processes. Physiological effects of salinity has been
302 demonstrated in the neighbouring, brackish Baltic Sea, where the low salinities have been shown to
303 critically affect survival and reproduction of copepods, notably *Pseudocalanus acuspes* (Möllmann
304 et al. 2003). However, the narrow range of salinities observed in this study (i.e. 34.7 to 35.2 PSU)
305 are well above the levels where salinity has any direct effect on reproduction. Instead, the opposite
306 responses to salinity demonstrated within both genera is likely related to the different geographical
307 origins of the water masses in this region (i.e. either Atlantic or coastal). Inflows of high salinity
308 and oxygen rich water from the Atlantic constitute the primary vector not only for nutrients
309 (Winther and Johanessen 2006) but also for passively drifting biota from source populations outside
310 the North Sea, including *C. finmarchicus*. This species advects into the North Sea from the north
311 after re-emerging from winter hibernation at great depths (Heath et al. 1999; Reid et al. 2003).
312 Hence, the positive salinity response of *C. finmarchicus* may largely reflect its Atlantic origin.

313 Whether a similar transport process can explain also the positive salinity response of *O. atlantica* is
314 unclear and merit further attention. In contrast to these species, *C. helgolandicus* and *O. similis*
315 show a clear preference for water masses with lower salinity. These water masses primarily
316 originate from coastal areas influenced by freshwater discharge from rivers (e.g. Rhine) and the
317 outflow from the brackish Baltic Sea. Due to the lower salinity (and higher temperatures) these
318 lighter water masses overlay the denser, saline and colder source water of more Atlantic origin. This
319 in turn may explain the more shallow distribution and high abundances of particularly *O. similis*
320 closer to the surface.

321

322 Species may not only reduce competition by occupying different physical environments but also by
323 shifting in opposite directions along a trade-off curve, such as that between maximizing foraging
324 and minimizing mortality. This may lead to their occurrence in patches with distinctly different
325 levels of both prey and predators. Despite different vertical distributions and affinities for
326 temperature and salinity all species show the same positive response of abundances to Chl *a*. This
327 indicates that all species concentrate (either by active movement or passive dispersal) and/or
328 perform better (i.e., in terms of higher egg production) in patches with high food availability, as has
329 previously been demonstrated for both *Calanus* and *Oithona* (Sabatini and Kiørboe 1994; Bonnet et
330 al. 2005; Ward and Hirst 2007; Melle et al., 2014). Consequently, differences in depth distribution
331 or environmental affinities does not necessarily reflect different priorities of the species in terms of
332 maximizing foraging opportunities. Nor does it reflect differences in feeding behaviour, since
333 species within each genus share the same feeding mode, i.e. either active, feeding current feeders
334 (*Calanus*) or passive, ambush feeders (*Oithona*). However, the differences in depth distribution may
335 reflect different prey preferences, for instance for different sized prey with distinct vertical
336 distributions, that are not captured by our crude proxy for food availability. Understanding such

337 differences in food availability and feeding preferences may shed light on the role and relative
338 importance of niche separation in terms of both food and the physical environment and the way in
339 which these differences determine the distribution and coexistence of species. One prominent
340 example is the clear niche separation between the ecologically similar species of sardine and
341 anchovy that coexist in upwelling areas around the world (Schwartzlose et al. 1999). Their niche
342 separation is manifested not only by different affinities for the physical environment (Takasuka et
343 al. 2007; Rykazewski and Checkley 2008; Lindegren et al. 2013) but also in terms of their
344 adaptations and preferences for food of different sizes (Van der Lingen et al. 2006).

345
346 Investment in foraging generally trades off against mortality, as higher feeding activity typically
347 increases exposure to potential predators. To minimize the risk of mortality, individuals may select
348 for habitats reducing predator exposure. For small planktonic organisms the primary behavioural
349 response is to avoid habitats with high light intensity where the probability of detection, at least by
350 visual predators, such as fish is increased. Although these trade-offs and mechanisms likely apply
351 also to our study species, the model results show no effect of light on the abundance distribution of
352 any of the species. This lack of statistical signal may point towards PAR being a poor proxy for
353 predation risk, as it does not represent the abundance of potential predators present in the area
354 during the time of sampling. However, light intensity has been shown to be a strong environmental
355 cue inducing various predator avoidance strategies among zooplankton, notably diel vertical
356 migration, reduced swimming and pigmentation (Bollens et al. 1994; Buskey et al. 2012; Lindegren
357 et al. 2012). Furthermore, small planktonic organisms such as copepods are unlikely able to directly
358 determine the abundance of predators in any given situation, unless they on the basis of strong
359 chemical cues are able to perceive and evaluate the local density of predators and consequently the
360 risk of predation (Heuschele and Selander 2014). Alternatively, the lack of a light effect may

361 indicate that by selecting for habitats with high food availability (as suggested by the positive Chl *a*
362 response) the individuals, regardless of species, may increase encounter rates of prey without
363 increasing feeding activity.

364

365 In conclusion, our results demonstrate pronounced spatial differences between species in each
366 genus, largely reflected in their vertical abundance distributions. These differences can primarily be
367 explained by different responses to the physical environment, notably in terms of their affinities for
368 habitats with different temperature and salinity conditions. Although we cannot rule out the
369 importance of biotic factors, including species-specific differences in prey preferences and the way
370 species may balance and optimize the trade-off between foraging and predation risk, our results
371 supports pronounced environmental niche separation among closely related copepod species in the
372 North Sea. Despite the relatively limited range of environmental conditions considered in our data
373 set and therefore our inability to fully characterize the entire width of the species environmental
374 niches, our results support the intuitive conclusions that niche separation is more pronounced
375 towards extreme environmental conditions where niche overlap is low (as illustrated by non-
376 overlapping confidence intervals of the fitted response curves; Fig. 4, 5). In terms of temperature
377 this occurs at <10-12° C, while at medium to high temperature the response curves converge and
378 niche separation is weaker, particularly for *Calanus*. Conversely, niche separation in terms of
379 salinity is pronounced >35 psu, while at medium and low salinities the niches and confidence
380 intervals largely overlap for both genera. We expect similar niche separation to occur also in high
381 diversity systems where multiple species (rather than pairs of species) occupy the same genus or
382 other functional grouping. Hence, we argue that environmental niche separation may act as a
383 primary mechanism reducing inter-specific competition and promoting coexistence and the
384 diversity of zooplankton species in marine and limnic ecosystems.

385 Finally, our study demonstrates the benefit of analysing ecological data using not a single but a
386 suite of different methods, including both traditional regression models and machine learning
387 approaches. By comparing results across methods, the sensitivity of outcomes to the choice of
388 method can formally be assessed. However, we fully acknowledge that applying multiple methods
389 can be challenging, especially since the application of machine learning approaches require a large
390 amount of data for training and cross-validation. Furthermore, machine learning tools are inherently
391 more opaque (e.g., due to the enormous amounts of decision trees produced), at least compared to
392 standard regression models providing readily interpretable parameter estimates. Nevertheless, in
393 our case all approaches (including GAM, RF and GBM) demonstrated similar statistical
394 relationships between species abundances and environmental drivers, hence supporting the
395 robustness of conclusions regarding the existence of environmental niches separation among closely
396 related zooplankton species.

397

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403

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Table 1. Variable importance of covariates, estimated as the percentage increase in the mean squared error of species abundances in the test data. This is calculated by permuting each covariate in the dataset, while retaining the existing model; an increase in the error after randomisation indicates that the variable was an important predictor within the model. Values without and within parenthesis show the estimates for each species and covariate based on RF and GBM, respectively.

	<u>C.fin</u>	<u>C.hel</u>	<u>O.atl</u>	<u>O.sim</u>
Temperature	44.1 (14.3)	53.9 (25.6)	46.7 (25.6)	54.8 (9.8)
Salinity	41.0 (37.8)	43.7 (2.9)	55.0 (57.1)	25.9 (0.1)
log(Chla)	27.9 (2.8)	47.0 (6.2)	35.0 (2.7)	34.5 (3.3)
log(PAR)	25.8 (0.7)	32.3 (0.2)	36.3 (1.4)	15.5 (0.1)
stage2	51.1 (17.7)	106.2 (57.4)	33.4 (12.9)	193.8 (78.5)
Transect	44.7 (26.7)	44.5 (7.8)	26.8 (0.3)	39.1 (8.2)

609 **Table 2.** Summary statistics of model performance for each species. The number of observations N,
610 the deviance explained of GAMs in %, the % variance explained pseudo-R² by random forest RF,
611 as well as the mean squared error MSE for each method, including gradient boosting machines
612 GBM.

613		GAM			RF		GBM
614	Species	N	DEV%	MSE	r ²	MSE	MSE
615	C. fin	298	53.1	1.31	63.9	1.01	1.81
616	C. hel	422	35.6	1.11	56.3	0.75	1.05
617	O. atl	310	36.8	1.71	59.5	1.08	2.12
618	O. sim	458	68.5	1.44	78.2	0.98	3.03

636 **Figure 1.** Map of the North Sea and the position of the transects included in the study: (A) Dogger
637 bank; (B) Buchanon and (C) Hanstholm.

638

639 **Figure 2.** The vertical distribution of log(Abundance) of (a) *Calanus finmarchicus* (blue) and
640 *Calanus helgolandicus* (red), as well as (b) *Oithona atlantica* (blue) and *Oithona similis* (red) at
641 each 10 meter depth interval from 5 to 65 meters. The box plots of species are arranged on top of
642 each other at every depth interval for visualisation purposes.

643

644 **Figure 3.** Boxplots of mean square errors (MSE) of model predictions based on 100 randomly
645 selected test data sets for (a) *Calanus finmarchicus*, (b) *Calanus helgolandicus*, (c) *Oithona*
646 *atlantica* and *Oithona similis* (d).

647

648 **Figure 4.** The relationships between temperature and the abundance distribution of (a) *Calanus* (*C.*
649 *finmarchicus*, blue; *C. helgolandicus*, red) and (b) *Oithona* (*O. atlantica*, blue; *O. similis*, red). The
650 left panels show the partial effects of temperature on log(Abundance) (centred at zero) based on the
651 final GAMs. Solid lines and shaded areas show the mean and 95% confidence intervals,
652 respectively, and filled circles the observed values for each species. The right panels show the
653 predicted log(Abundances) (transformed to zero mean and unit variance) based on RFs (solid lines)
654 and GBM (dashed lines).

655

656 **Figure 5.** The statistical relationships between salinity and the abundance distribution of (a)
657 *Calanus* (*C. finmarchicus*, blue; *C. helgolandicus*, red) and (b) *Oithona* (*O. atlantica*, blue; *O.*
658 *similis*, red). The left panels show partial effect plots of log(Abundances) (centred at zero) based on
659 the final GAMs where solid lines and shaded areas show the mean and 95% confidence intervals,

660 respectively and filled circles the observed values for each species. The right panels show the
661 predicted log(Abundances) (transformed to zero mean and unit variance) based on random forest
662 (solid lines) and GBM (dashed lines).

663

664 **Figure 6.** The statistical relationships between log(Chlorophyll a) and log(abundances) of (a,b)
665 *Calanus* (*C. finmarchicus*, blue; *C. helgolandicus*, red) and (c,d) *Oithona* (*O. atlantica*, blue; *O.*
666 *similis*, red). The left panels show partial effect plots of log(abundances) (centred at zero) based on
667 the final GAMs where solid lines and shaded areas show the mean and 95% confidence intervals,
668 respectively and filled circles the observed values for each species. The right panels show the
669 predicted log(Abundances) (transformed to zero mean and unit variance) based on random forest
670 (solid lines) and GBM (dashed lines).

671

672 **Figure 7.** Predicted environmental niches of *C. finmarchicus* (a), *C. helgolandicus* (b), *O. atlantica*
673 (c) and *O. similis* (d) across the entire range of observed temperature, salinity and Chlorophyll a
674 values. The size of the dots correspond to the predicted log(Abundances) for each species based on
675 GAMs and illustrate the relative abundance distribution across the 3-D environmental niche space.

676

1 **SUPPLEMENTARY MATERIAL**

2 **Table S1.** Model selection based on likelihood ratio tests and the Generalized Cross Validation (GCV) scores for each GAM. The total
 3 deviance explained (DEV in %), the significance of the likelihood ratio test (p) and the number of observations (n) are shown. The final
 4 models are highlighted in bold.

5	Response	Model	GCV	DEV(%)	p	n
6	1.	$\log(\text{C.fin}) \sim \alpha + s(\text{Temp}) + s(\text{Sal}) + s(\log(\text{Chl a})) + s(\log(\text{PAR})) + \text{transect} + \text{stage}$		1.4064	53.8	
7	2.	$\log(\text{C.fin}) \sim \alpha + s(\text{Temp}) + s(\text{Sal}) + s(\log(\text{Chl a})) + \text{transect} + \text{stage}$	1.4009	53.6	0.29	
8	3.	$\log(\text{C.fin}) \sim \alpha + s(\text{Temp}) + s(\text{Sal}) + \text{transect} + \text{stage}$	1.399	53.1	0.18	298
9	1.	$\log(\text{C.hel}) \sim \alpha + s(\text{Temp}) + s(\text{Sal}) + s(\log(\text{Chl a})) + s(\log(\text{PAR})) + \text{transect} + \text{stage}$		1.1649	36.2	
10	2.	$\log(\text{C.hel}) \sim \alpha + s(\text{Temp}) + s(\text{Sal}) + s(\log(\text{Chl a})) + \text{transect} + \text{stage}$	1.163	36.0	0.25	
11	3.	$\log(\text{C.hel}) \sim \alpha + s(\text{Temp}) + s(\text{Sal}) + \text{transect} + \text{stage}$	1.1641	35.6	0.13	422
12	1.	$\log(\text{O.atl}) \sim \alpha + s(\text{Temp}) + s(\text{Sal}) + s(\log(\text{Chl a})) + s(\log(\text{PAR})) + \text{transect} + \text{stage}$		1.8256	37.1	
13	2.	$\log(\text{O.atl}) \sim \alpha + s(\text{Temp}) + s(\text{Sal}) + s(\log(\text{Chl a})) + \text{transect} + \text{stage}$	1.8317	36.4	0.09	300
14	1.	$\log(\text{O.sim}) \sim \alpha + s(\text{Temp}) + s(\text{Sal}) + s(\log(\text{Chl a})) + s(\log(\text{PAR})) + \text{transect} + \text{stage}$		0.702	71.0	
15	2.	$\log(\text{O.sim}) \sim \alpha + s(\text{Temp}) + s(\text{Sal}) + s(\log(\text{Chl a})) + \text{transect} + \text{stage}$	0.701	70.9	0.29	419

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Table S2. Summary statistics of parametric coefficients and smooth terms for the final GAM for *C. fincmarchicus*. edf is the estimated degrees of freedom for the model smooth terms where edf>1 indicates a non-linear relationship.

Parametric coefficients	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	2.1501	0.1599	13.448	< 2e-16 ***
Stage:Female	-0.6296	0.1494	-4.215	3.34e-05 ***
Stage:Male	-1.6822	0.2079	-8.091	1.67e-14 ***
Transect 2	-1.0724	0.1996	-5.374	1.59e-07 ***
Transect 3	0.2602	0.2706	0.962	0.337
Transect 4	1.0428	0.1975	5.280	2.55e-07 ***
Smooth terms	edf	Ref.df	F	p-value
s(Temp)	1.950	1.997	28.479	4.25e-12 ***
s(Sal)	1.802	1.961	7.085	0.00112 **
R-sq.(adj) = 0.517 Deviance explained = 53.1%				
GCV = 1.399 Scale est. = 1.3533 n = 298				

Table S3. Summary statistics of parametric coefficients and smooth terms for the final GAM for *C. helgolandicus*. edf is the estimated degrees of freedom for the model smooth terms where edf>1 indicates a non-linear relationship.

Parametric coefficients	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	2.58782	0.11818	21.897	< 2e-16 ***
Stage:Female	0.02966	0.12521	0.237	0.812894
Stage:Male	-1.41341	0.13349	-10.588	< 2e-16 ***
Transect 2	0.38518	0.14776	2.607	0.009470 **
Transect 3	1.06772	0.27266	3.916	0.000105 ***
Transect 4	0.80616	0.21149	3.812	0.000159 ***
Smooth terms	edf	Ref.df	F	p-value
s(Temp)	1.992	2.000	20.535	3e-09 ***
s(Sal)	1.763	1.944	4.646	0.011 *
R-sq.(adj) = 0.343 Deviance explained = 35.6%				
GCV = 1.1641 Scale est. = 1.1372 n = 422				

79 **Table S4.** Summary statistics of parametric coefficients and smooth terms for the final GAM for *O.*
80 *atlantica*. edf is the estimated degrees of freedom for the model smooth terms where edf>1
81 indicates a non-linear relationship.

82 Parametric coefficients	Estimate	Std. Error	t value	Pr(> t)
83 (Intercept)	3.11378	0.21960	14.180	< 2e-16 ***
84 Stage:Female	-0.63899	0.19228	-3.323	0.001001 **
85 Stage:Male	-0.72853	0.19794	-3.681	0.000276 ***
86 Transect 2	-0.39187	0.20688	-1.894	0.059168 .
87 Transect 3	-0.02362	0.30985	-0.076	0.939274
88 Transect 4	-1.11610	0.41270	-2.704	0.007236 **
89 Transect 5	-2.32136	1.25341	-1.852	0.065008 .

91 Smooth terms	edf	Ref.df	F	p-value
92 s(Temp)	1.447	1.694	8.238	0.000938 ***
93 s(Sal)	1.590	1.832	11.476	4.17e-05 ***
94 s(log(Chl a))	1.413	1.654	5.339	0.009515 **

95 R-sq.(adj) = 0.346 Deviance explained = 36.8%
96 GCV = 1.8472 Scale est. = 1.7789 n = 310

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109 **Table S5.** Summary statistics of parametric coefficients and smooth terms for the final GAM for *O.*
 110 *similis*. edf is the estimated degrees of freedom for the model smooth terms where edf>1 indicates a
 111 non-linear relationship.

112					
113	Parametric coefficients	Estimate	Std. Error	t value	Pr(> t)
114	(Intercept)	6.9631	0.2350	29.629	< 2e-16 ***
115	Stage:Female	-0.5670	0.1861	-3.046	0.00246 **
116	Stage:Male	-3.3366	0.1868	-17.864	< 2e-16 ***
117	Transect 2	-1.4363	0.1800	-7.979	1.26e-14 ***
118	Transect 3	-0.7095	0.3046	-2.329	0.02029 *
119	Transect 4	-0.3553	0.2060	-1.725	0.08530 .
120	Transect 5	0.4899	0.6371	0.769	0.44231
121					
122	Smooth terms	edf	Ref.df	F	p-value
123	s(Temp)	1.948	1.997	25.493	3.15e-11 ***
124	s(Sal)	1.696	1.908	3.046	0.051147 .
125	s(log(Chla))	1.000	1.000	12.549	0.000437 ***

126 R-sq.(adj) = 0.677 Deviance explained = 68.5%

127 GCV = 1.5158 Scale est. = 1.4772 n = 458

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142 **Figure S1.** Vertical profiles of *Calanus* (left column) and *Oithona* (right column) species for five
143 selected field series. The size of symbols show the relative abundances of given species (absolute
144 abundances can differ for species in same graph) while type and color indicate the species where
145 red/square represents *C. finmarchicus* and *O. atlantica* and blue represents *C. helgolandicus* and *O.*
146 *similis*. The contour lines show levels of salinity while the solid grey area indicates the bottom.
147 Depth (m), distance indicated by station number, with 10 nm between stations.
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Figure S2. The vertical distribution of log(Abundances) of (a) *Calanus finmarchicus*, (b) *Calanus helgolandicus*, (c) *Oithona atlantica* and (d) *Oithona similis* by each life-stage corresponding to adult females (light grey) and males (dark grey) and juveniles(i.e., copepodites, black). Dashed smooth lines illustrate the main vertical distribution for each species and life-stage.

157

158 **Figure S3.** Residual diagnostics for the final GAM for *C. fincmarchicus*. The axis-labels referring

159 to fitted values, response and linear predictor are in the unit of log(abundances).

160

161 **Figure S4.** Residual diagnostics for the final GAM for *C. helgolandicus*. The axis-labels referring

162 to fitted values, response and linear predictor are in the unit of log(abundances).

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165 **Figure S5.** Residual diagnostics for the final GAM for *O. atlantica*. The axis-labels referring to

166 fitted values, response and linear predictor are in the unit of log(abundances).

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 169 **Figure S6.** Residual diagnostics for the final GAM for *O. similis*. The axis-labels referring to fitted
 170 values, response and linear predictor are in the unit of log(abundances).